

Autosensitization to DNA: Evidence for an Immunologic Basis

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A 59-year-old female with spontaneous painful ecchymoses developed ecchymoses after intracutaneous injection of washed autologous whole blood cells and calf thymus DNA. Immunofluorescent studies of the spontaneous lesions revealed granular deposits of IgM, C3, factor B and properdin at the dermal-epidermal junction but no deposits in her normal skin. T cells were decreased in number but responded normally to polyclonal mitogens and did not transform in response to DNA-containing antigens. Repair of UV-damaged DNA by her lymphocytes appeared to be depressed. The findings presented here are the first immunologic abnormalities uncovered in this disorder and may help in understanding the pathogenesis of the inflammatory lesions seen in autosensitization to DNA.

DNA in its native state is not antigenic in normal animals. However, in patients with systemic lupus erythematosus (SLE), there may be circulating antibodies to DNA [1] as well as skin test reactivity to injected DNA [2]. A rarer disorder, autosensitization to DNA, is characterized also by skin test reactivity to injected DNA, but clinical presents as recurrent painful ecchymoses without other systemic manifestations. Since 1961 when Levin and Pinkus first reported a patient with this syndrome, the pathophysiology has remained obscure [3].

We report a patient with characteristic clinical findings, skin test reactivity to DNA and the first laboratory evidence to suggest an immunologic basis for the skin lesions in autosensitization to DNA. Comparisons are also made between this syndrome, autoerythrocyte sensitization and SLE.

CASE REPORT

FK, a 59-yr-old female, presented in the outpatient clinic at the Arizona Health Sciences Center in 1972 with painful ecchymoses of her face, arms and legs of 4 mo duration. The lesions suddenly and spontaneously appeared on her palms, lip, chin, arms and legs. On 2 occasions there was pharyngeal involvement with swelling and mild respiratory distress. The lesions began as warm, red, raised, tender nodules, 0.5 to 2.0 cm in diameter, which initially felt numb as swelling occurred, but progressed over several hours to larger, painful ecchymoses. Lesions began in one area, but soon appeared in crops in other areas. Individual lesions usually cleared within 48 hr but entire episodes lasted from 4 to 10 days and occurred at 1 to 2 week intervals.

She had taken diethylstilbestrol for 2 mo prior to the onset, but its discontinuation had no effect. There was no renal or joint involvement,

no previous easy bruisability, nor a family history of similar illness. Aside from the ecchymoses and several carious teeth, physical examination was unremarkable.

The CBC, differential, white blood count, platelet count, prothrombin time, partial thromboplastin time, serum protein electrophoresis, BUN, urinalysis and urinary porphyrins were negative. Chest and sinus radiographs and an electrocardiogram were negative. During attacks of ecchymoses the Westergren erythrocyte sedimentation rate was 42 and 48 ml/hr. Complement studies during 2 episodes shows mildly decreased CH50 values, but normal levels of C3, C4, C5 by quantitative radial immunodiffusion. On 4 other occasions all complement studies, including CH50 were normal. Assay for C1q immune complexes, a measure of circulating immune complexes, was negative. C1 esterase inhibitor level was normal. A tourniquet test was followed by heat and swelling of her forearm which persisted for 72 hr. Antinuclear antibody determinations on 2 occasions showed trace patchy nuclear strain at 1:4 dilution, but was negative on four other occasions. LE prep was negative. Studies for antibody to mitochondria, smooth muscle and parietal cells were also negative.

Exclusion of aspirin, food dyes, preservatives and a variety of foods as well as extraction of carious teeth had no effect. Therapeutic trials with chlorpheniramine, cyproheptadine and hydroxychloroquine were without effect. She was maintained on prednisone, varying from 5 mg on alternate to 30 mg each morning which appeared to abort the episodes. Finally, she developed angina pectoris, was placed on propranolol, and her lesions decreased to the point where steroids were discontinued and there have been no exacerbations for more than 1 yr.

MATERIALS AND METHODS

Skin Test Materials

Autologous leukocytes were separated from heparinized blood by dextran sedimentation, washed 3 times in sterile saline, suspended at 10^6 cells per ml, then lysed by 10 cycles of alternate freezing in carbon dioxide snow and thawing at 37° after the method of Schwartz, Lewis, and Dameshek [4]. After removing the leukocyte-rich plasma, erythrocytes were washed, suspended in saline, and lysed [4]. Autologous white blood cells were washed 3 times in saline but not lysed prior to injection.

Calf thymus DNA (Worthington Biochemicals, Freehold, N. J.) was dissolved in 0.01 M phosphate buffered 0.15 M NaCl, pH 7.4 (PBS) containing 0.003 M $MgCl_2$ by agitation at $4^\circ C$ for 72 hr. A 1 mg/ml solution was passed through 0.45 μ pore-size micropore filter and placed in a sterile vial for testing.

Anti-DNA Antibodies

Radioimmunoassays for antibodies to native DNA, heat-denatured DNA, deoxyribonucleoprotein and circulating free DNA were performed as described previously [5]. Radioallergosorbent test (RAST) for antibodies to native DNA and heat-denatured DNA were kindly performed by Dr. Donald Hoffman, Department of Pathology, East Carolina University, Greenville, North Carolina, as described elsewhere [6,7].

Skin Biopsies

Four-millimeter punch biopsies were taken from the upper arm with red, warm, raised lesions which had not yet progressed to ecchymoses, as well as from normal skin. A biopsy of 1 lesion was sectioned in 2, one-half placed in 10% formalin, and the other snap-frozen in Tissue Tec (Ames Laboratories, Elkhardt, Indiana) for frozen sections. Four micrometer sections were cut at $-20^\circ C$, placed on glass slides and air-dried.

Immunofluorescent Studies

Direct fluorescent antibody studies were performed using fluoresceinated antihuman C3 and antihuman immunoglobulin antisera as

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Abbreviations:

Con A: concanavalin A

RAST: radioallergosorbent test

SLE: systemic lupus erythematosus

described previously [8]. Rabbit antihuman properdin was prepared as described previously [9]. Rabbit antihuman factor B and C4 were kindly supplied by Dr. H. Muller-Eberhard. Antiserum to UV-irradiated DNA was prepared in rabbits and UV treatment of sections was prepared as described elsewhere [10]. Fluoresceinated sheep antirabbit globulin was purchased from Meloy Laboratories, Springfield, Virginia and absorbed twice with normal human serum, 2 parts conjugate to 1 part serum. Indirect immunofluorescence was performed as follows: 10 min PBS wash, specific rabbit antiserum incubation of section for 30 min, 10 min PBS wash, sheep antirabbit fluoresceinated antiserum incubation of section, 10 min PBS wash, and finally 50% glycerol in PBS before mounting with a glass coverslip.

Guinea pig lip and normal skin were used as substrates over which serum was layered to test for circulating antibasement membrane antibodies. Indirect immunofluorescence was performed as outlined above using rabbit antihuman immunoglobulin fluoresceinated antiserum.

Controls for the specificity of conjugated antisera to immunoglobulin and C3 were performed by absorption of the conjugates with unconjugated immunoglobulins or C3 which abolished their staining as described previously [8]. Absorbed fluoresceinated sheep antirabbit globulin produced no staining when applied to sections directly or to sections previously incubated with unconjugated normal rabbit serum.

Cellular Studies

T cells were enumerated by counting sheep erythrocyte [E] rosettes and B cell surface immunoglobulin distribution was evaluated by direct immunofluorescence as outlined elsewhere [11].

Lymphocyte transformation studies were carried out in triplicate using phytohemagglutinin (Difco Laboratories, Detroit, Michigan), pokeweed mitogen (Grand Island Biologicals, Grand Island, New York) and concanavalin A (Con A) (Pharmacia Laboratories, Piscataway, New Jersey) at concentrations of 20,200 and 200 mg per ml as described elsewhere [12]. Calf thymus DNA was prepared in native, heat-denatured and UV-altered forms as described elsewhere [5,10] and used in concentrations of 0.5, 1.0, 5.0, 25 and 125 mg/ml in lymphocyte transformation as outlined elsewhere [13].

UV-DNA repair was performed by a method previously described [14] as follows: Lymphocytes were obtained and suspended in culture medium with hydroxyurea for synchronization in G-1 of cell cycle. Cells were irradiated at 25.6 nm and returned to culture with tritiated thymidine. The uptake of label was measured and compared to aliquots of unirradiated cells and cells from normal individuals treated in an identical manner.

Photography

Fluorescent antibody slides were examined on a Leitz Ortholux microscope with a darkfield condenser. Polaroid ASA 3000 film was used for exposure periods of 45 to 90 seconds [8].

RESULTS

At 15 min after intracutaneous injection of autologous whole blood, lysed leukocytes and calf thymus DNA into the patient, wheal and flare responses appeared at these sites. At 4 hr, painful red, raised lesions appeared, enlarging until 24 hr at which time painful ecchymotic lesions developed, which diminished in size and faded over the ensuing 96 hr. In contrast, a control individual who received identically treated autologous blood cells developed only a pale red, nontender area 8 mm in diameter by 24 hr which disappeared within 3 days. When the control individual received calf thymus DNA, the maximum size of the induced lesion was 5 mm in diameter at 4 hr which subsided by 24 hr. However, in the patient, calf thymus DNA produced a 10 mm in diameter wheal at 15 min, followed by a 20 mm in diameter, tender, indurated lesion which began to diminish by 24 hr when an ecchymosis appeared. The skin test results are summarized in Table I.

Radioimmunoassays for circulating antibodies to native DNA and to deoxyribonucleoprotein were negative and no free DNA was found in the serum. RAST studies for specific IgE antibodies to native DNA and heat-denatured DNA were negative.

Immunofluorescent studies of the skin lesions revealed stippled fluorescence at the dermal-epidermal junction for C3, IgM, properdin and factor B (see Figure, a-e). No deposits of IgG, IgA, or C4 were found. Irregular, lumpy deposits were most

TABLE I. Intracutaneous tests

Injected material	Time after injection			
	Wheal	Induration		
		15 min	4 hr	18 hr
Washed blood cells	2+ ^a	3+	3+	4+
Lysed erythrocytes	Neg	Neg	Neg	Neg
Lysed leukocytes	2+	2+	2+	1+
Calf thymus DNA	2+	2+	2+	1+
Saline control	Neg	Neg	Neg	Neg

^a 1+ = 3-5mm, 2+ = 6-10mm, 3+ = 11-20mm, 4+ = >20mm.

striking for C3 and IgM (Figure, a and b). Sections of normal skin from this patient were negative by immunofluorescence. Routine histologic examination of an early lesion revealed solar elastosis but otherwise was negative.

Tissue sections of lesions irradiated with ultraviolet light and stained for DNA using rabbit anti-UV-DNA antiserum revealed nuclei in the epidermis and scattered throughout the dermis, some nuclear debris, but no definite deposits of DNA at the dermal-epidermal junction (Figure, f). If DNA had been present in large amounts in this region, UV-irradiation would be expected to produce a thymidine-dimer photoproduct detectable with rabbit antiserum to UV-DNA. Studies for circulating antibasement membrane antibodies using patient's serum with guinea pig lip and and normal human skin as substrates failed to show fluorescence at the dermal-epidermal junction.

Lymphocyte studies revealed that T cells comprised only 11% of the total as detected by E rosettes (normal 60-70%), and were as follows: IgG 15%, IgM 10%, IgA 5%, Kappa 15% and Lambda 12%. Lymphocyte transformation studies showed normal responses to PHA, PWM and Con A. Preparations of N-DNA, H-DNA and UV-DNA failed to stimulate increased thymidine incorporation by lymphocytes.

Evaluation of DNA repair capacities of UV irradiated lymphocytes demonstrated that normal individuals show 5.3 times the level of incorporation of tritiated thymidine in irradiated cells whereas cells from the patient had depressed repair of UV-damaged DNA with an uptake of only 2.5 times above unirradiated aliquots. This result is identical to those obtained with cells from patients with SLE (see Table II).

DISCUSSION

This patient's illness has many features similar to those reported in the rare disorder, autosensitization to DNA [3,4,15,16]. Her positive skin tests to autologous washed blood cells, lysed leukocytes and calf thymus DNA reflect dermal sensitivity to DNA [4]. The immediate wheal and flare skin test reactivity observed in this patient is characteristic of chemical mediator release which can be induced by antigen combining with IgE antibody on mast cells or basophils, but complement activation via the classical or alternative pathway can generate anaphylatoxins which also can trigger mediator release mimicking IgE-mediated reactions [17]. One would expect antibodies of the IgE class to have been detected by RAST and antibodies of other classes to have been detected by a primary binding radioimmunoassay, but these studies were negative. Biopsy sections studied by immunofluorescence contained IgM and complement at the dermal-epidermal junction but the antigen could not be demonstrated. Therefore, only meager evidence exists for these reactions being antibody-mediated.

With regard to a cellular basis for the reactions, lymphocytes responded normally to polyclonal mitogens PHA, Con A and PWM but did not transform when exposed to DNA in several forms including native DNA, single-stranded DNA and UV-irradiated DNA. Normal controls responded in a similar manner. The patient's lymphocytes were not entirely normal, however, and certain abnormalities were demonstrated including a large null cell population lacking surface markers, and depressed repair of UV-damaged DNA [14]. In SLE, there also

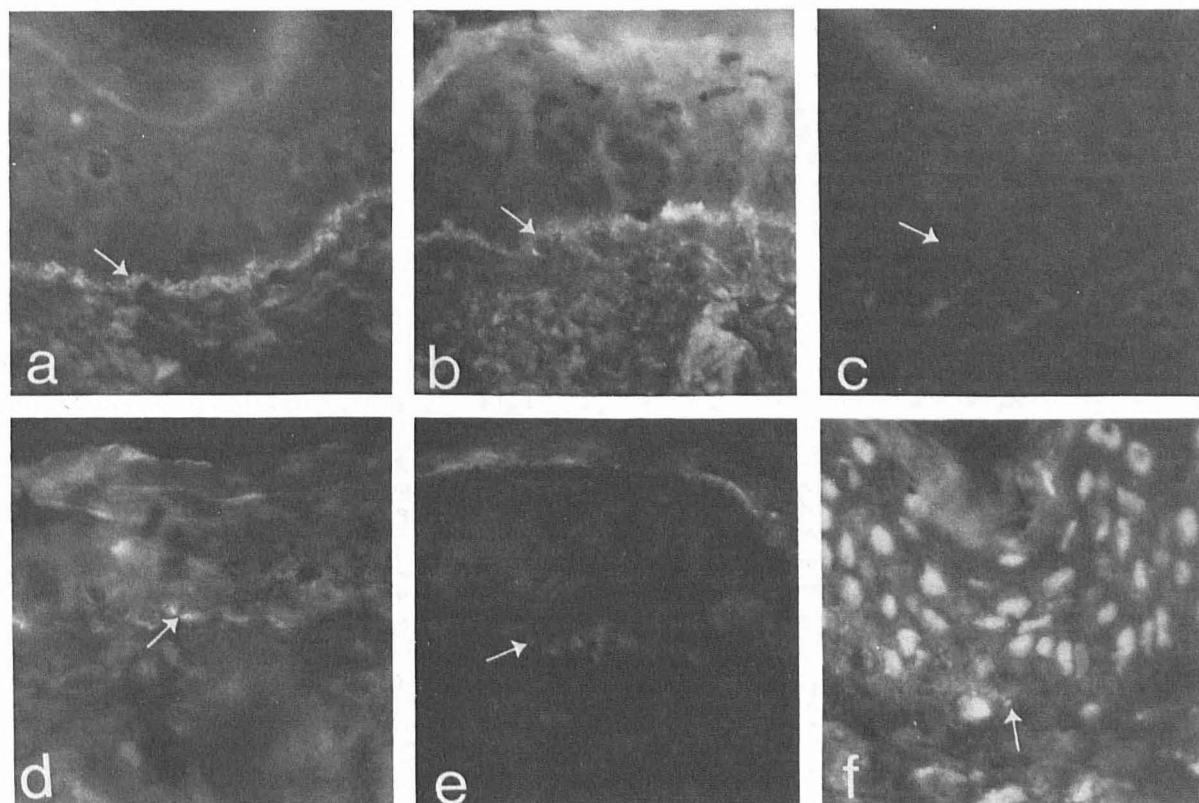


FIG 1. Fluorescent photographs of sections of lesion from patient with autosensitization to DNA ($\times 400$). There is granular staining along dermal-epidermal junction for (a) C3 (b) IgM (c) C4 is negative (d) factor B (e) properdin are positive (f) UV-DNA is detected in nuclei in epidermis and some nuclear debris to left of arrow. There is nonspecific stain of keratin and dermal layers. Arrows point to dermal-epidermal junction.

TABLE II. Post-UV labeling of lymphocytes synchronized in G-1 of cell cycle

Subjects	No. in group	X-Fold increase (mean)	
Autosensitization to DNA	1	2.5	
SLE	12	2.5	} $p < 0.01$
Controls	20	5.3	

may be a large null cell population [19], and depressed repair UV-damaged DNA [14], but cellular responses to polyclonal mitogens are usually depressed [12] and responses to DNA may be increased [13] compared with controls.

Several findings in this patient bear similarities to previously reported findings in SLE including: (1) Skin test reactivity to autologous leukocytes and calf thymus DNA [2] (2) decreased numbers of T cells and a large null cell population [19] (3) deposits of immunoglobulin and complement at the dermal-epidermal junction in a skin lesion [18] (4) defective repair of UV-damaged DNA in lymphocytes [14].

The presence of immunoglobulin and complement at the dermal-epidermal junction suggests the presence of immune complexes but the antigen could not be demonstrated. Whether DNA might be present but masked by antibody or somehow degraded *in situ* is not known. Antibody to the dermal-epidermal junction itself also could not be demonstrated. The finding of IgM, with C3, factor B and properdin along the dermal-epidermal junction without C4 being demonstrated is of interest. Factor B and properdin are components of the alternative pathway which typically may be activated by carbohydrate surfaces or immunoglobulin aggregates [20]. C3 can be activated by either the classical or alternative pathway, following which there is activation of later components C5 through C9. IgM is capable of producing cell damage via the alternative pathway

utilizing a system described with C4-deficient guinea pig serum or C2 deficient guinea pig serum in which activation of a C1-like protein is associated with conversion of factor B [21]. A similar bypass mechanism involving IgM activation of the alternative pathway might be operative in our patient but this is purely speculative. Activation of the classical as well as the alternative pathway has been reported in SLE and several other diseases [22]. The normal level of C3 in our patient does not exclude activation since C3 is an abundant protein with a rapid turnover rate [23]. Mild depression of CH50 with normal serum levels of C3 does not exclude skin fixation of C3 since synthetic processes might be able to compensate for complement utilization.

The differential diagnosis in this form of nonthrombocytopenic purpura includes autoerythrocyte sensitization, purpura factitia, vasculitic purpura and SLE. Autoerythrocyte sensitization was described by Gardner and Diamond in 1955 as a form of chronic purpura which appeared to follow an injury associated with extravasation of blood [24]. Their patients reacted with ecchymoses to injected autologous whole blood cells autologous erythrocytes and erythrocyte stroma. Most patients with autoerythrocyte sensitization have had bizarre behavior and hypnosis could reproduce the lesions in some so that the designation "psychogenic purpura" has been suggested for this syndrome [25]. In 1 patient both native DNA and erythrocytes could induce ecchymoses and C3 was depressed, but one cannot be sure that this study was truly double-blind [26]. Our patient was not neurotic and reacted to injected DNA but not to erythrocytes so a diagnosis of autoerythrocyte sensitization or psychogenic purpura was not made.

Purpura factitia, an apparently self-inflicted disease [27], is not a likely diagnosis since some lesions appeared in relatively inaccessible areas and saline skin tests were negative with the patient unaware as to which sites received saline or test materials.

Patients with necrotizing angiitis or vasculitis of the skin with palpable purpura have had abnormal complement profiles in certain instances but these abnormalities have correlated best with the underlying disease such as SLE, Sjogren's syndrome, or rheumatoid arthritis [28]. Nonatopic patients with urticaria and chronic cutaneous vasculitis refractory to treatment had accompanying arthralgias or arthritis with or without hypocomplementemia [28]. Our patient did not have arthralgias, nor vasculitis on skin biopsy.

With regard to the differential diagnosis between SLE and autosensitization to DNA it must be emphasized that there was no renal or joint involvement or LE cell positivity in our patient and the present case does not meet the proposed diagnostic criteria for SLE [29].

It is not clear how DNA, autologous and heterologous, induced immediate and late skin test reactions in this patient, but polynucleotides, as well as DNA or partially degraded DNA, can react with C1q [30]. DNA, presented properly, might activate complement with or without the participation of antibody or perhaps material with such a large molecular size could alter skin permeability directly. Positive skin tests to DNA which have been reported in SLE [2] also are not a diagnostic criterion for SLE. Furthermore, our patient lacked antibodies to native DNA or deoxyribonucleoprotein which are characteristic of SLE [5].

The depressed repair of UV-damaged DNA observed in this patient is in the range described for SLE [14] but the defect is much more marked in xeroderma pigmentosum in which virtually no uptake is found following cell irradiation [31]. Depressed repair of UV-irradiated DNA, although similar to the defect observed in lymphocytes from SLE patients, is not sufficient evidence to propose autosensitization to DNA as a form of SLE since the test is relatively new and has been used only in a limited number of disease states. It is tempting to speculate that defective DNA repair in autosensitization to DNA and in SLE could generate immunogenic material, possibly overcoming the capacity of nucleases to degrade DNA, but further studies would be needed to confirm this.

In summary, in this patient with autosensitization to DNA, the demonstration of immunoglobulin and complement components along the dermal-epidermal junction, decreased numbers of T cells and depressed repair of UV damaged DNA suggests that an immunologic process contributes to the generation of the skin lesions in this syndrome. Although a clinical diagnosis of SLE cannot be made, there are several common features in these 2 disorders so one cannot exclude the possibility that autosensitization to DNA may represent a variant of SLE.

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